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by

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NUTRIENT REQUIREMENTS AND BIOLOGICAL SOLIDS ACCUMULATION IN ANAEROBIC DIGESTION

Richard E. Speece
Perry L. McCarty

Anaerobic digestion as known by the sanitary engineer is a unique and hardy process and has proved to be a powerful tool in the stabilization of strong organic wastes. Practically any type of organic matter can be decomposed to methane and carbon dioxide as the principal end products with very little in the way of toxic by-products being produced. As Buswell[3] has stated, methane fermentation can be carried on in a mixed or enriched culture and hence it is possible to maintain the process on a large scale continuously for apparently an indefinite period.

Primarily, in the past, anaerobic digestion has been applied to treatment of complex insoluble wastes, such as sewage sludge. While feeding such heterogenous wastes, the biological solids accumulation has been obscured due to the presence of significant amounts of organic matter which are very resistant to biological stabilization. In conventional activated sludge and trickling filter processes, the biological solids accumulation is more apparent since the settleable solids are removed from the waste stream prior to introduction in the aeration tank or on the filter. Thus, only soluble and colloidal organic matter is biologically treated and the synthesis resulting during treatment is approximated by the wasted sludge from the secondary treatment process.

In evaluating a treatment process, it is important to know the degree of biological synthesis as three undesirable effects result from synthesis of organic wastes into biological solids; (1) provision must be made for additional treatment of these solids, (2) synthesis converts simple soluble compounds into more complex, more difficult to degrade biological solids, and (3) synthesis of cellular protoplasm requires a source of nitrogen which increases treatment costs for nitrogen deficient wastes.

The synthesis relationships have been established for many classes of substrates undergoing aerobic stabilization, since it has been possible to feed pure substrates along with the normal inorganic salts and achieve satisfactory aerobic treatment. However, unsatisfactory operation has usually been experienced

in the anaerobic digestion of pure and relatively simple substrates when these were the sole organic source. The difficulty experienced in the pure culture isolation of methane bacteria has also retarded the development of fundamental knowledge concerning the anaerobic digestion process.

The conversion of simple organic compounds to methane and carbon dioxide by the methane bacteria is a vital key to the successful operation of the process. Consequently, if this link fails the whole process is endangered. Unsatisfactory digestion has usually been attributed to failure of the methane bacteria to process the simple organic compounds. Therefore, it is important to study the process and determine the growth requirements and synthesis relationships in order to more fully utilize the excellent potentialities of anaerobic digestion as an organic waste treatment method and also to place process design on a stronger scientific basis.

Synthesis Relationships

Heukelekian, et al,[7] and Weston and Eckenfelder[19] have used a growth equation of the general form shown in equation (1), in connection with aerobic systems.

$$A = cF - kM \qquad (1)$$

where:

A = sludge accumulation, lb/day
c = constant, fraction of substrate synthesized
F = load, lb BOD or lb COD utilized/day
k = constant, endogenous respiration rate/day
M = volatile solids present in system, lbs.

This equation has proven to be a reliable expedient in engineering design for estimating sludge production from a biological treatment process. Heukelekian, et al,[7] established the following equation from laboratory data of activated sludge processes treating domestic sewage:

$$A = 0.5\ F - 0.055\ M$$

Retaining the same nomenclature as above, the load, F, was based on BOD utilized.

Weston and Eckenfelder[19] analyzed data of other investigators and plotted A/M vs F/M values to show the relationship between BOD removed and activated sludge produced. They thought a definite ratio should exist between BOD removed, the fraction oxidized and the fraction synthesized for any given set of conditions, but that in practice it is difficult to determine these relationships exactly because cellular material is oxidized concurrently with that of the waste.

Monod[11] said that bacterial growth experiments generally give cell yields proportional to the amount of substrate available. With a particular organism and substrate, the yield was constant for all growth rates and substrate concentrations, and varied only slightly with temperature. Therefore, the same basic principles which control synthesis in aerobic processes would be expected to govern anaerobic processes.

Stewart, et al,[17] applied basic principles in the formulation of a rational approach to the design of continuous flow anaerobic systems and evaluated a growth equation for a particular anaerobic system. Experimentally, they used a substrate composed of Bacto-Tryptone, dextrose, and Bacto-Beef extract and reported the growth equation for this particular substrate undergoing anaerobic digestion to be:

$$A = 0.18\ F - 0.0247\ M$$

They based the load, F, on substrate COD utilized. These investigators stated that other processes with different substrates and organism cultures will probably have different process reaction constants.

Growth Requirements in Anaerobic Digestion

Barker[1] studied the methane fermentation of acetate extensively and made the following statement:

> "The methane bacteria have not been studied as extensively as most other groups of bacteria of comparable scientific and practical importance. The reason is readily apparent. In order to study the biology and biochemistry of bacteria most effectively it is necessary to use pure cultures. Unfortunately, with methane bacteria this elementary but basic requirement has been difficult and in many instances impossible to achieve."

This gives an insight into the primitive state of knowledge concerning methane bacteria, in spite of the fact that they play such a strategic role and are so widely utilized in biological waste treatment processes. A number of investigators have reported results which indicate the need of a growth-promoting substance for methane bacteria.

Heukelekian and Heinemann[6] conducting experiments on the seeding of digesters, concluded that the chief value of digested sludge lies in the production of favorable environmental conditions and not in the numbers of bacteria added. This observation may also indicate the presence of growth-promoting substances in the digested sludge.

Stander[15] studied the possibility of treating winery waste by anaerobic digestion. He found it necessary to periodically add sludge from a digester fed raw sewage sludge. Without these periodic additions, the winery waste could not be digested at high loading rates. This indicates some necessary growth-promoting substance was present in the digested sludge but not in the pure winery waste.

McCarty and Vath[10] reported that digestion of acetic acid alone could not proceed at high rates if only the normal inorganic salts were present. They found high rates of acetate digestion required the presence of some substance contained in the supernatant liquor solids from a domestic sewage sludge digester.

In this study unsatisfactory digestion developed with substrates in all categories as the original seed sludge was purged from each digester, the common denominator of difficulty being a gradual increase in the volatile acid concentration which decreased only slightly even when feeding was stopped. Thus, conditions affecting fermentation of acetate affected digestion of practically all the organic wastes.

Preliminary Study on Stimulation of Acetate Digestion

In view of the reports of other investigators and the difficulty encountered in this study, it was necessary to carry out a preliminary study to determine some pure compounds which would promote satisfactory acetate digestion in the absence of the heterogenous sludge used for initiating digestion. It was hoped that such compounds could then be included with the daily feed to the digesters where biological solids accumulation was being studied, enabling satisfactory digestion, and yielding reliable data for each substrate.

A procedure similar to that used by McCarty and Vath[10] was followed in this preliminary study. An attempt was made to operate the digesters under conditions such that only the lack of stimulatory compounds limited the rate of acetate utilization. It was endeavored to maintain all other conditions as nearly ideal as possible. Continuous gas recirculation insured that the digester contents were uniformly mixed. A sufficiently low buffer concentration was maintained to avoid ion toxicity. The temperature was closely controlled at $35 \pm 0.5^{\circ}C$ to prevent the microorganisms from being thermally shocked, and the substrate was continuously fed at approximately the rate at which it was being utilized, while maintaining a reserve volatile acid concentration between 1000

and 1500 mg/l. There were occasions, however, when both the volatile acids concentration and the normal inorganic salts were found to be limiting the maximum rate of acetate utilization. Whenever such a deficit occurred, it was remedied as soon as possible.

The inorganic salts were initially maintained at about the following concentrations within the digester:

$NaHCO_3$	-	5000 mg/l
$(NH_4)_2 HPO_4$	-	230 mg/l
$Mg SO_4$	-	10 mg/l
$NH_4 Cl$	-	280 mg/l
K Cl	-	40 mg/l
$Mg Cl_2$	-	50 mg/l

These concentrations, excepting that of $NaHCO_3$, were subsequently tripled, since they were found to be limiting acetate utilization at increased levels of stimulation. Sufficient Cambridge, Massachusetts tap water, containing most trace elements required for bacterial growth was added to the substrate and inorganic salts solution to bring the total volume to 200 ml, which, when fed to the 6-liter digesters, gave a 30-day theoretical detention time. A constant volume was maintained within the digesters by daily wasting of about 200 ml of the mixed contents.

The schematic layout of the digesters is shown in Figure 1. Initially the digesters were seeded with screened, digested primary sludge obtained from the Nut Island Sewage Treatment Plant in Boston, Massachusetts. Operation of the digesters involved analysis of a sample of the wasted contents for volatile acids concentration, calculation of the feed dosage of acetate which would satisfy the acetate utilization rate and maintain the volatile acids concentration between 1000 and 1500 mg/l. Gas production was metered and recorded to provide a check on acetate utilization. Periodically, solids concentration and ammonia and organic nitrogen were determined on the effluent. Continuous operation was carried on until less than 1% of the original seed sludge remained. The accompanying acetate utilization rates had decreased to about 300 mg/l/day.

Under these latter conditions, actual assay of pure chemical compounds for stimulation potential in acetate digestion was commenced. Some of the compounds chosen were reported to be growth requirements for certain types of bacteria. These compounds were then added to the acetate digesters and their stimulation potential assayed by the change in rate of acetate utilization.

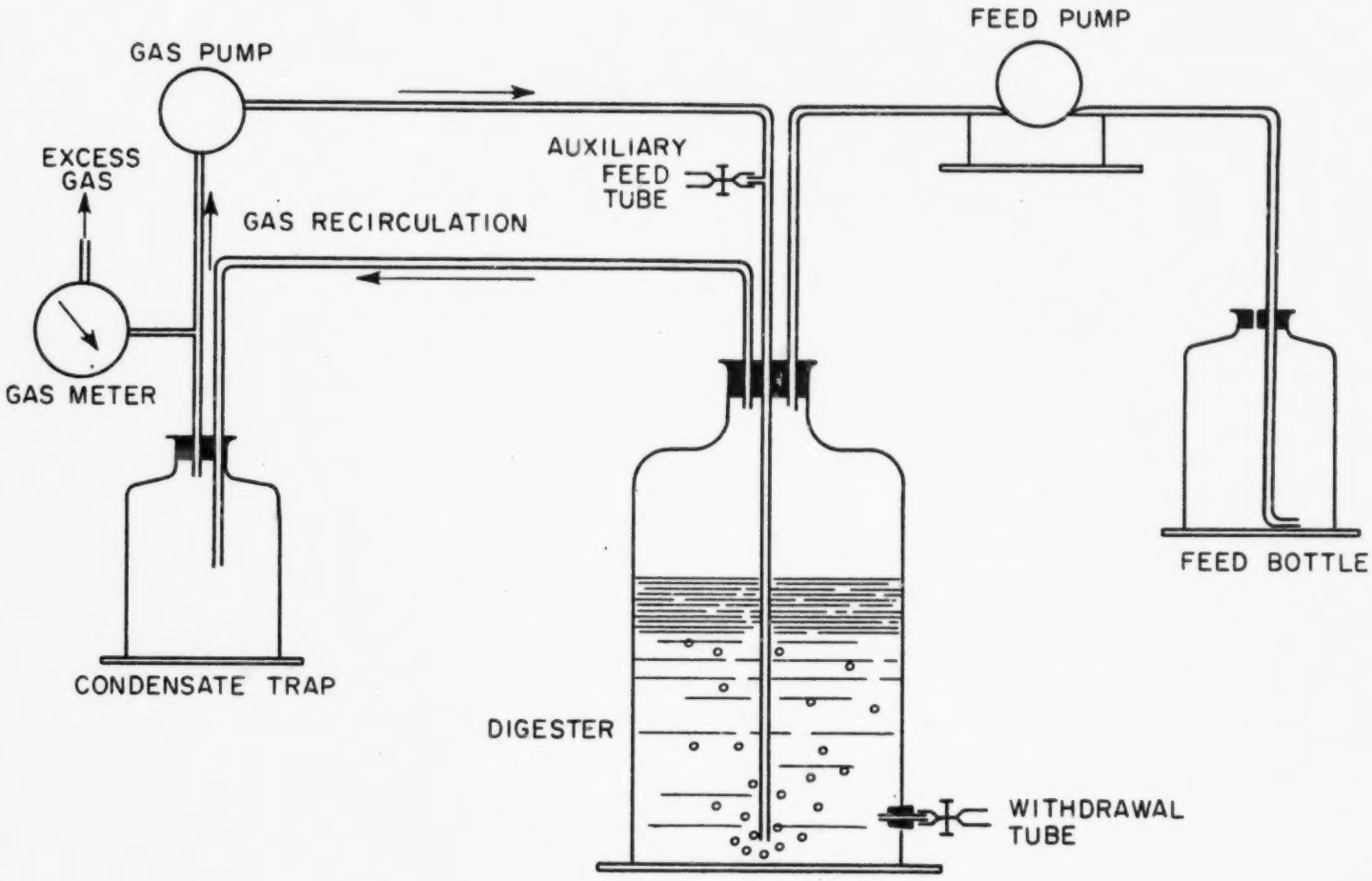

Figure 1 Continuous Feed Digester System

Results of Preliminary Study on Stimulation of Acetate Digestion

A number of pure compounds were assayed for stimulation potential. Those compounds which yielded positive stimulation are listed below along with the increase in acetate utilization rates which resulted.

Compound	Concentration	Rate Increase
Thiamine	20 mg/l	1400 mg/l/day
Proline	50 mg/l	500 mg/l/day
Glycine	40 mg/l	1000 mg/l/day
Benzimidazole	15 mg/l	800 mg/l/day
$CoCl_2$	20 mg/l	2400 mg/l/day
$FeCl_3$	500 mg/l	2300 mg/l/day

Glycine, proline, benzimidazole and cobalt implicated vitamin B_{12} as a stimulatory compound. Glycine appears as an intermediary product in the synthesis of a portion of the vitamin B_{12} molecule, porphyrin. Cobalt is the particular ion attached at the center of porphyrin for vitamin B_{12}. Individual units of porphyrin are similar to the proline molecule. Also, vitamin B_{12} contains a benzimidazole unit as part of its structure. However, addition of vitamin B_{12} with an intrinsic factor of mannitol failed to stimulate acetate digestion.

Since the intermediate metabolic steps in the splitting of the acetate molecule to yield methane and carbon dioxide have not yet been defined, it is premature to attempt to explain the stimulatory function of these compounds. Combinations of the above stimulatory compounds were found to increase acetate digestion rates from 700 to 6500 mg/l/day in a digester purged of essentially all the original seed sludge. Sufficient information was obtained to fulfill the requirements for this particular investigation on biological synthesis and nitrogen and phosphorous requirements. However, additional studies will yet be required to evaluate and confirm the need for any or all of the above stimulants and to determine the maximum extent of stimulation that can be achieved by their presence.

Procedure

Biological Solids Accumulation

The biological solids accumulation and consequent nitrogen and phosphorus requirements were determined for the anerobic digestion of a number of organic compounds. The category and substrates chosen were:

Fats:	Acetate
	Octanoate
	Oleate
Proteins:	Glycine
	Leucine
	Nutrient Broth
Carbohydrates:	Glucose
	Starch
	Cellulose

These categories of organic compounds are commonly encountered in biological waste treatment processes. The substrates in each category were chosen because it was considered each group would closely represent all the various compounds likely to be encountered in that particular category. Fatty acids were chosen to represent the fats category since the glyceride unit (fat) is hydrolyzed to yield glycerol and fatty acids. Acetate, octanoate, and oleate represent short, medium, and long chain fatty acids, respectively. The protein class presented a problem, since it is difficult to choose "representative" compounds due to the varied and complex nature of the molecule encountered in this group. However, since proteins are composed of amino acids, it was decided to choose two amino acids and a common complex proteinaceous material. Glycine, leucine, and nutrient broth were thus chosen. No precipitation of substrate was found to occur in the digesters receiving any of these three compounds. This was important, for precipitation of proteinaceous substrate would have obscured cellular organic nitrogen data by addition of non-cellular solids containing organic nitrogen. For the carbohydrate substrates, glucose, starch, and cellulose were chosen, since they represent a gradation in size and complexity of molecule and are commonly encountered.

In an attempt to simulate field conditions of operation, a series of digesters was set up as shown in Figure 2. The apparatus was enclosed in a constant temperature room maintained at $35 \pm 0.5^{o}C$ to correspond to the mesophilic range of digestion most commonly encountered in practice. Also, a closed atmosphere of gases produced in the digestion process was maintained to eliminate the toxic effect of oxygen. With this apparatus, it was possible under rigid anaerobic conditions to measure the gas production volume at atmospheric pressure, waste a sample of the digester contents, expel the gas accumulation, and introduce the feed solution. The loading rate at which all substrates were fed was 0.04 $lb/ft^3/day$ (670 mg COD/l/day), to simulate conventional digestion loading, and the contents were thoroughly mixed once a day. All substrates were fed on at least two sludge retention times with a minimum of 5 and a maximum of 30 days.

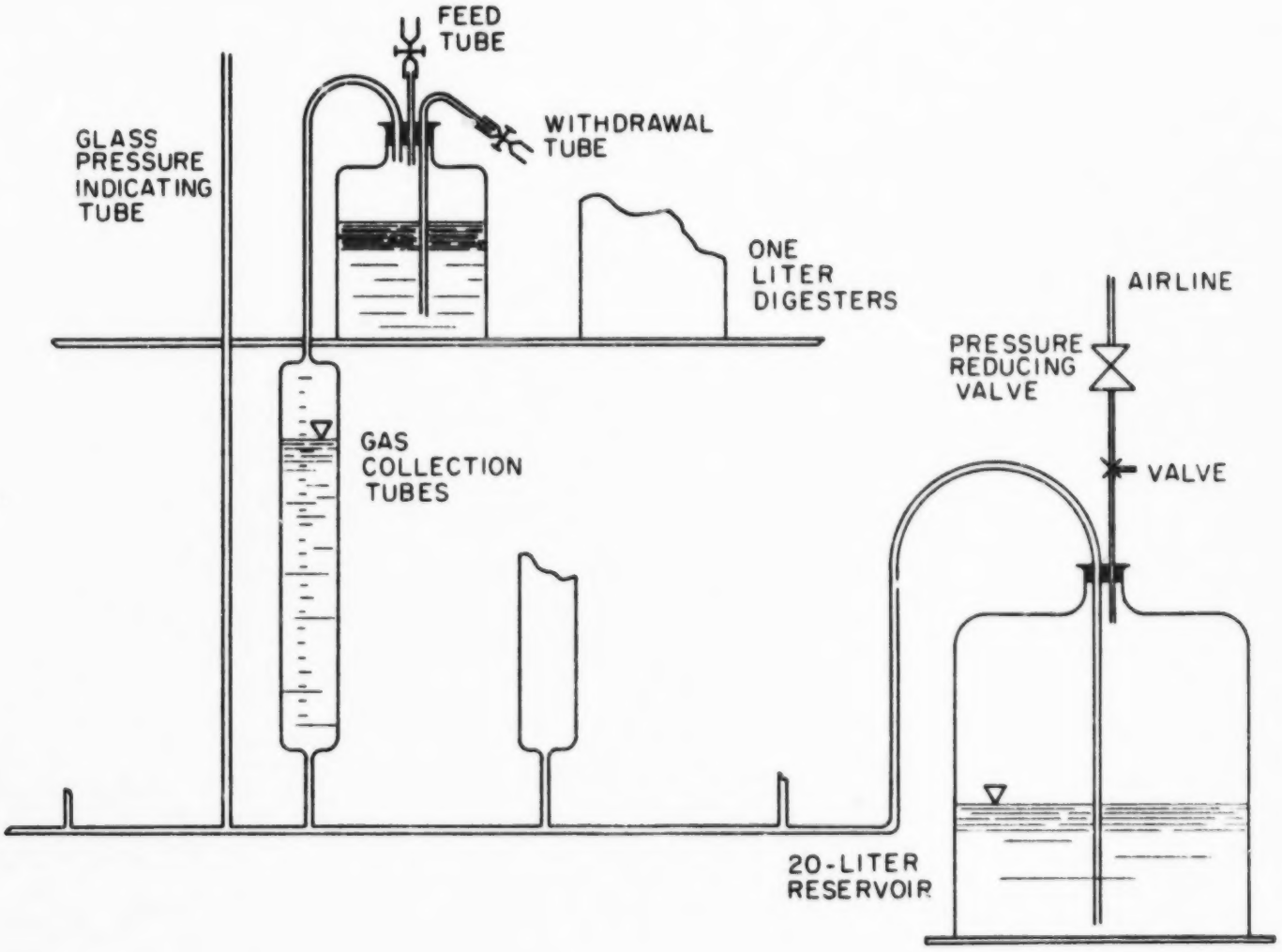

Figure 2 Batch Feed Digester System

The digesters were initially seeded with primary digested sludge from the Nut Island Sewage Treatment Plant in Boston, Massachusetts. This sludge was first passed through 1/8" mesh screen to remove coarse material and paper. One-fourth liter was then transferred to each digester and 500 ml of warm tap water was added to dilute to the required 750 ml. It was necessary that the digester be purged of this original seed sludge so that analyses of the digester contents would reflect only biological solids. The purging operation was accomplished by daily withdrawal of a sample of the uniformly mixed digestion contents and replacing this with the pure substrate and necessary inorganic salts. By this procedure, less than 1% of the original seed sludge remained after 4 detention periods. After this time, all digesters were inverted and the last vestiges of sediment were allowed to settle in the digester mouth and were then washed out by expelling a portion of the digester contents. Therefore, after that time, analyses of the contents of a digester reflected only biological activity resulting from utilization of the particular substrate fed to that digester.

All digesters were operated until equilibrium conditions prevailed as indicated by consistent organic nitrogen concentrations in the effluent and gas production records. Final data was recorded over an interval of equilibrium operation of not less than ten days and up to forty-five days, to observe any fluctuations that might have been encountered over extended periods. No significant variations were noted during these extended periods of equilibrium operation.

The sludge retention time was the same as the hydraulic detention time in this study, since uniformly mixed samples of the digester contents were expelled daily. Thus, the sludge retention time was controlled by varying the quantity of dilution water added to the daily feed. A fraction of the mixed digester contents was expelled, corresponding to the feed volume, prior to the daily feeding operation to maintain a constant volume.

The daily feed to each digester consisted of 500 mg COD of the particular substrate, and Cambridge, Massachusetts tap water was added to dilute the feed to the required volume. Based on the study of stimulation of acetate digestion, the following concentrations of inorganic salts were added to the digesters operating on fatty acid and protein substrates, while it was found that these concentrations had to be tripled in the carbohydrate digesters due to the increased synthesis which occurred:

230 mg/l	$(NH_4)_2\ HPO_4$
10 mg/l	$MgSO_4$
280 mg/l	NH_4Cl
40 mg/l	KCl
50 mg/l	$MgCl_2$
100 mg/l	$FeCl_3$

Of the list of stimulants from the acetate stimulation study, it was found necessary only to add ferric chloride along with the normal inorganic salts to obtain satisfactory digestion at the chosen loading rate after essentially all of the seed sludge had been purged. The buffer capacity was maintained in the digesters receiving fatty acids and carbohydrate substrates by adding sodium becarbonate in the feed at a concentration of approximately 6000 mg/l. This addition was not required in the proteinaceous substrate digesters as sufficient buffer was produced by the ammonium bicarbonate end product. Here, it was necessary to neutralize with hydrochloric acid the excess ammonium bicarbonate alkalinity created in the digestion of the proteinaceous substrates operating on the longer sludge retention times. Otherwise, the resulting increase in pH led to ammonium toxicity which inhibited digestion, as reported by McCarty and McKinney[9].

The following analyses were made regularly on samples of the digester contents: volatile acids concentration, COD of the mixed contents, COD of the solids-free liquor, organic nitrogen of the mixed contents (for the protein substrates the organic nitrogen was determined on the centrifuged and washed solids), ammonia nitrogen, alkalinity, pH, suspended and volatile suspended solids. The gas from all digesters was analyzed for methane, carbon dioxide, and nitrogen content.

Microchemical analyses were run on the centrifuged and washed biological solids, to determine the empirical chemical cell formulation in terms of carbon, hydrogen, oxygen, nitrogen, and phosphorous. This was performed in the Microchemical Analyses Laboratory of the Massachusetts Institute of Technology.

Organic nitrogen served as the basis for nitrogen requirement calculations. Phosphorous requirements were determined by microchemical analysis of the effluent solids. Exceptions in microchemical analysis determinations were the cellulose and oleate digesters which contained insoluble forms of the substrate and thus digester solids would not be representative of bacterial cells, and were excluded from the microchemical analyses.

RESULTS

Biological Solids Accumulation

The operating characteristics were determined for the anaerobic digestion of the chosen substrates for at least two different sludge retention times, with the exception of oleate. In this case reliable data could be collected for operation on only a 30-day retention time. Also, due to unsatisfactory digestion with leucine and cellulose at 5 days sludge retention time, operation was switched to 7.5 days where successful digestion was maintained.

Table 1 summarizes the operating data from all the runs. The substrate and the sludge retention time on which the digesters were operated are listed in Columns 1 and 2. Column 3 records the number of days for which the digesters were operated after equilibrium conditions had been reached, and during which final data was recorded. The gas production was recorded daily and the gas was periodically analyzed to determine its composition. The methane fraction of total gas production was then used to derive the actual methane production recorded in Column 4.

Uniformly mixed samples of the digester contents were drawn daily prior to feeding. For the digesters receiving fatty acid and carbohydrate substrates, the mixed effluent were analyzed for organic nitrogen. Since no organic nitrogen was contained in these feeds, the organic nitrogen analysis reflected cellular nitrogen and enzymes secreted into the solution. On several occasions, organic nitrogen analyses were run on the centrifuged and washed solids. The results were essentially the same as on the mixed liquor and indicated that enzyme concentrations in solution were negligible. For the digesters receiving protein substrates, it was necessary to centrifuge and wash the effluent solids in order to insure that the organic nitrogen analyses included no traces of undecomposed substrate, which also contained organic nitrogen. This would have obscured the actual organic nitrogen concentration due to biological solids. Cellular organic nitrogen concentrations in the respective digesters are shown in Column 5.

The nitrogen requirements as shown in Column 6 were calculated as follows:

$$\text{Nitrogen Requirement} = \frac{\text{Cellular Organic Nitrogen in mg/l}}{(\text{Sludge Retention Time in Days})\ (\text{Loading Rate in gm COD/l day})\ (\text{Efficiency})}$$

For the acetate digester operating on a 5-day sludge retention time, a sample calculation is given:

$$\text{Nitrogen Requirement} = \frac{12 \text{ mg/l}}{(5 \text{ days})\ (0.670 \text{ gm COD/l/day})(0.67)}$$

$$= 5.3 \text{ mg N/gm COD utilized}$$

Column 7 lists the average volatile solids concentration. This value was not obtained for the digesters operating on acetate. The feeding of oleate and cellulose gave rise to the presence of insoluble, non-cellular organic matter within the digesters, therefore volatile solids analyses of the contents were not determined. The net synthesis in Column 8 was calculated as follows:

$$\text{Net Synthesis} = \text{Nitrogen Requirement} \times 11.4$$

The factor 11.4 was found to be the proportion of COD to cellular nitrogen in this study as will be shown later.

Removal efficiencies shown in Column 10 were based upon the COD concentration in the feed and on the average COD of the solids-free effluent. One exception to this was the acetate substrate, where efficiences were based upon volatile acid concentrations in the feed and in the effluent. Corrections were made to the COD of the effluents from the oleate and cellulose digesters. With these substrates, the COD of the mixed effluent was determined, and reduced by a factor of 11.4 times the cellular nitrogen concentration to correct for the inclusion of bacterial cells with particulate undecomposed substrate.

As a check on the reliability of the data, a balance was made on the COD input to the system and the COD output from the system. The feed was the only source of COD input, while methane and the effluent sample were the two sources of COD output. The COD of the mixed effluent sample was determined, and the total daily gas production and methane composition recorded. The volume of methane at 35°C which is equivalent to 1 gram of COD is theoretically calculated to be 395 ml. The COD balance was made for the entire final data period and is recorded in Column 11.

Figure 3 is a plot of nitrogen requirements as shown in Table 1, Column 6 versus sludge retention time. This reveals graphically, the difference in biological growth which can be expected during the digestion of various types of organic wastes, since nitrogen requirements were directly proportional to net synthesis of substrate into biological solids.

TABLE I

SUMMARY OF DIGESTER OPERATION

Substrate	Sludge Retention Time (Days)	Days Operated at Equilibrium	Daily CH_4 Product. mℓ	Cell-N Conc. mg/ℓ	N-Reqm't mg N/gm COD	Volatile Solids mg/ℓ	Net Synthesis of Substrate COD Utilized,%	Removal Efficiency %	COD Balance %
Acetate	5	45	143	12	5.3	--	6.0	67	111
Acetate	10	45	186	23	3.7	--	4.2	93	106
Acetate	15	45	205	36	3.7	--	4.2	98	110
Acetate	20	45	202	40	3.0	--	3.4	99	106
Acetate	25	45	206	46	2.8	--	3.2	99	108
Acetate	30	45	198	54	2.7	--	3.1	99	104
Octanoate	5	25	115	11	6.1	104	6.9	65	101
Octanoate	30	25	171	53	2.8	492	3.2	96	94
Oleate	30	25	175	51	2.7	--	3.1	95	94
Glycine	5	20	137	11	4.0	78	4.5	83	108
Glycine	30	13	156	55	2.9	468	3.3	93	88
Leucine	7.5	10	105	20	5.6	180	6.4	70	88
Leucine	30	17	156	54	2.8	400	3.2	95	86
Nutrient Broth	5	17	130	22	7.5	190	8.5	87	90
Nutrient Broth	30	17	126	104	5.8	700	6.6	89	82
Glucose	5	45	77	81	37	620	42	65	112
Glucose	10	45	114	130	22	990	25	86	99
Glucose	20	40	135	216	17	1970	19	93	96
Glucose	30	30	117	215	12	2080	14	89	84
Starch	5	10	49	60	36	500	41	50	101
Starch	30	10	152	293	15	2200	17	97	98
Cellulose	7.5	15	90	79	23	--	26	68	92
Cellulose	30	21	137	162	9	--	10	94	76

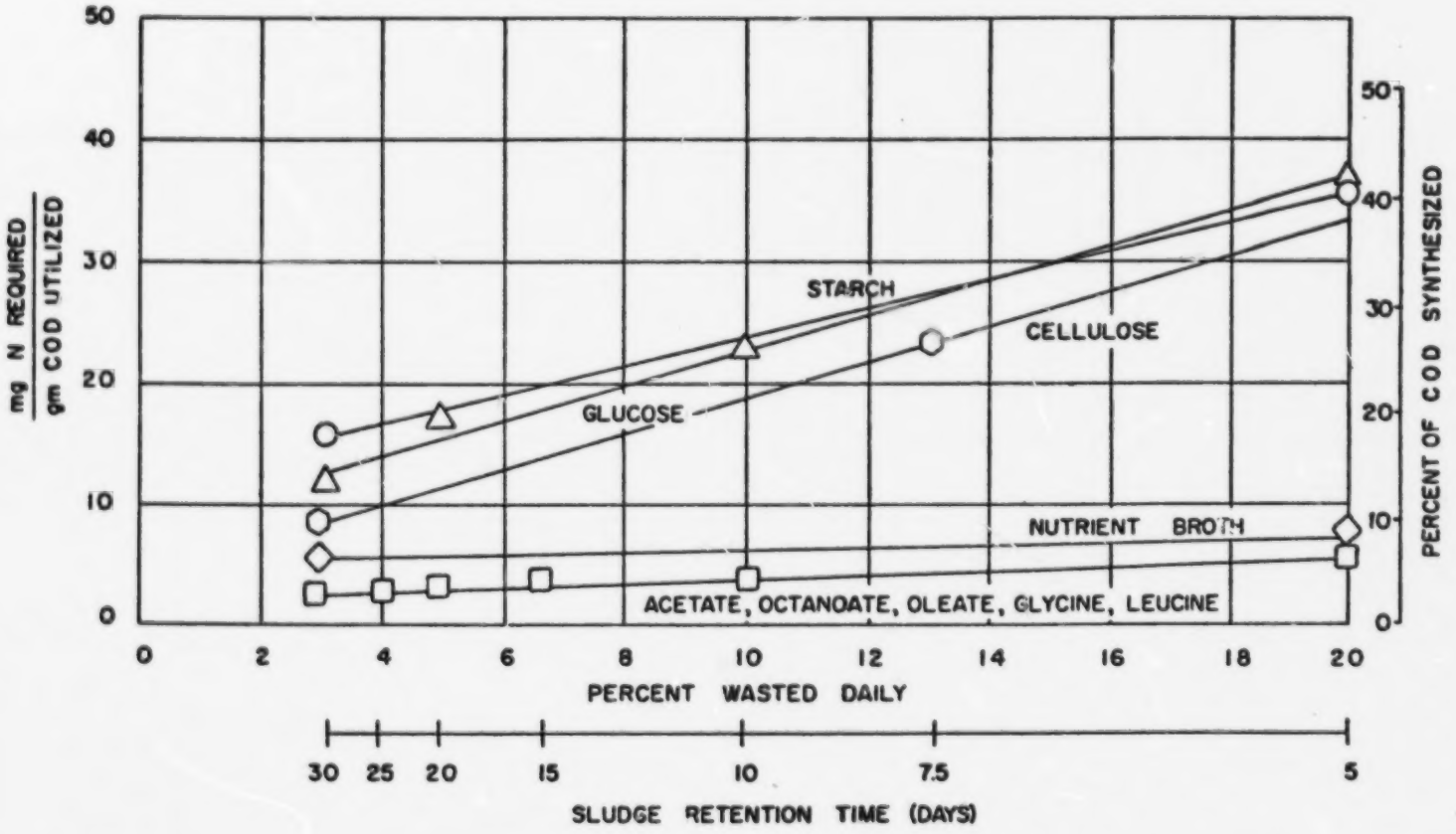

Figure 3 NITROGEN REQUIREMENTS

Chemical Formulation of Bacterial Protoplasm

Microchemical analyses of the biological solids responsible for the anaerobic digestion of all substrates, except the insoluble oleate and cellulose, were determined. Table II shows the chemical formulation of these biological solids for the various substrates and their respective sludge retention times. The N/P ratio in the biological solids, based on weight, is given. Also, the COD/N ratio is listed, as calculated theoretically from the empirical formula.

The average of all the empirical formulations in Table II yielded the formula $C_{4.8}H_{8.8}O_{2.9}N$. For simplicity, the subscripts were rounded off to the nearest whole number to yield the general empirical formula of anerobic biological solids as found in this study:

$$C_5H_9O_3N$$

This compares closely with empirical formulations of biological solids produced aerobically. Symons and McKinney[18] reported a formula of $C_5H_8O_2N$, while Hoover and Porges[8] reported $C_5H_7O_2N$.

The COD/N ratio for the general empirical formula, $C_5H_9O_3N$, is 11.4, while the cell weight/N ratio is 9.4. These ratios were verified experimentally in the course of this study by data taken on volatile solids, COD, and cellular nitrogen of the biological solids. The N/P ratio appears to be approximately 7. All of the respective values averaged together for the general empirical formula fell within ± 2 standard deviations of the mean. There appears to be no statistical difference between the general formula and any of the individual formulas.

Evaluation of Growth Equation Constants

Since the biological solids and cellular nitrogen are interdependent, as shown experimentally in this study and also stated by Bennett and Williams[2], the growth equation constants, c and k, can be evaluated on the basis of either net synthesis of volatile solids or nitrogen requirements. It was decided to use the latter basis, since these data were available for all substrates. Therefore, the cellular nitrogen concentrations were multiplied by 9.4, the ratio between volatile solids and cellular nitrogen as determined in this study, to obtain representative volatile solids concentrations.

The data were grouped as follows: the amino and fatty acids; glucose and starch; and nutrient broth by itself. This grouping was chosen, since the nitrogen requirements, as shown in Figure 3, indicated this was a logical division. The constants were not evaluated for cellulose alone since the daily wasting schedule of operation was 7.5 and 30 days sludge retention time (13% and 3%). This did not represent a very wide spread of points and would have magnified any error involved.

The growth equation constants were evaluated as shown in Table III and Figures 4, 5 and 6. The data are plotted in the form $A/F = c - kM/F$, which is the straight line equation form ($y = mx + b$) of the growth equation. The values A, F, and M were measured experimentally. The constant c is the y-axis intercept and k is the slope of such a plot.

The line of best fit for the groups of amino and fatty acids, and glucose and starch was determined by the method of least squares. Since only two points of data were available for nutrient broth, the line of best fit could only connect these points:

The growth equations were evaluated to be:

Amino and fatty acids	$A = 0.054F - 0.038M$
Glucose and starch	$A = 0.46F - 0.088M$
Nutrient broth	$A = 0.076F - 0.014M$

The loading rate, F, in these equations was based upon COD reduction between the influent and effluent filtrate of the respective digesters.

DISCUSSION

Biological Solids Accumulation

The results of this phase of the investigation have elucidated the biological response which occurs in the anaerobic digestion of each of the three classes of substrates studied. The most significant observation from this phase of the research was the discovery of the exceptionally high net synthesis and correspondingly reduced methane production from carbohydrates which occurs at short sludge retention times. Thirty to forty percent of the carbohydrate COD was converted to biological solids COD during anaerobic digestion at short sludge retention times as compared to 6 - 8 % conversion for substrates in the fats and proteins class under similar conditions. This observation has not been reported hitherto and merits a note of caution from those responsible for process design of waste treatment facilities.

TABLE II

CHEMICAL FORMULATION OF BIOLOGICAL SOLIDS

Substrate	Sludge Retention Time (Days)	Empirical Formula					Ash (%)	N/P Wt. Basis	COD/N
		C	H	O	N	P			
Acetate	5	4.9	11.3	3.5	1	--	56	--	12.0
Acetate	10	5.1	10.4	3.1	1	--	35	--	12.4
Acetate	15	4.9	9.4	2.9	1	--	37	--	11.6
Acetate	20	4.3	8.3	2.2	1	--	32	--	10.4
Acetate	25	5.4	9.4	3.4	1	--	28	--	12.2
Acetate	30	5.0	11.0	2.7	1	0.03*	24	7	12.9
Octanoate	5	4.6	6.9	2.4	1	0.05	9	9	10.1
Octanoate	30	4.7	7.7	2.1	1	0.04	5	12	11.1
Oleate	30	--	--	--	-	--	--	--	--
Glycine	5	4.8	7.6	3.0	1	0.06	8	8	10.2
Glycine	30	4.9	9.0	3.0	1	0.11	11	4	11.2
Leucine	7.5	5.0	8.8	3.2	1	0.05	9	9	11.1
Leucine	30	4.1	7.6	3.3	1	0.05	5	9	8.0
Nutrient Broth	5	4.1	6.8	2.2	1	0.04	10	12	9.1
Nutrient Broth	30	3.8	7.0	2.8	1	0.05	16	9	7.8
Glucose	5	4.8	8.3	2.5	1	0.17	20	3	11.6
Glucose	10	5.1	8.5	2.5	1	0.14	14	3	12.0
Glucose	20	5.9	10.1	3.2	1	0.18	11	2	13.8
Glucose	30	4.9	10.2	3.1	1	0.21	26	2	11.8
Starch	5	5.0	8.7	2.9	1	0.09	6	5	11.5
Starch	30	5.3	9.1	2.5	1	0.06	6	8	12.6
Cellulose	7.5	--	--	--	-	--	--	--	--
Cellulose	30	--	--	--	-	--	--	--	--

*Phosphorous value determined on solids from another acetate digester utilizing 4800 $\frac{\text{mg COD}}{\ell \text{ day}}$

TABLE III

EVALUATION OF GROWTH EQUATION CONSTANTS

Substrate	Sludge Retention Time (Days)	Cellular Nitrogen (mg/ℓ) M_n	Biological Volatile Solids (mg/ℓ) $M=9.4 \times M_n$	Volatile Solids Accumulation Per Day (mg) A	Removal Efficiency %	F COD Utilized Per Day (mg/ℓ)	A/F mg Solids Accumulation Per mg COD Utilized/ℓ	M/F mg Solids Per mg COD Utilized/ℓ
Acetate	5	12	113	23	67	450	0.051	0.25
Acetate	10	23	218	22	93	620	0.036	0.35
Acetate	15	36	339	23	98	650	0.035	0.52
Acetate	20	40	376	19	99	660	0.029	0.57
Acetate	25	46	433	17	99	660	0.026	0.66
Acetate	30	53	500	17	99	660	0.026	0.76
Octanoate	5	11	103	21	65	430	0.049	0.24
Octanoate	30	54	508	17	96	640	0.027	0.79
Oleate	30	53	500	17	95	640	0.027	0.78
Glycine	5	11	103	21	83	560	0.037	0.18
Glycine	30	55	517	17	93	620	0.027	0.83
Leucine	7.5	20	188	25	70	470	0.053	0.40
Leucine	30	54	508	17	95	640	0.027	0.79
Nutrient Broth	5	22	207	41	87	580	0.071	0.36
Nutrient Broth	30	104	975	32	89	600	0.053	1.63
Glucose	5	81	761	152	65	430	0.35	1.77
Glucose	10	131	1230	123	86	580	0.21	2.12
Glucose	20	216	2030	102	93	620	0.16	3.27
Glucose	30	215	2020	67	89	600	0.11	3.37
Starch	5	60	565	113	50	330	0.34	1.71
Starch	30	293	2750	92	97	650	0.14	4.22

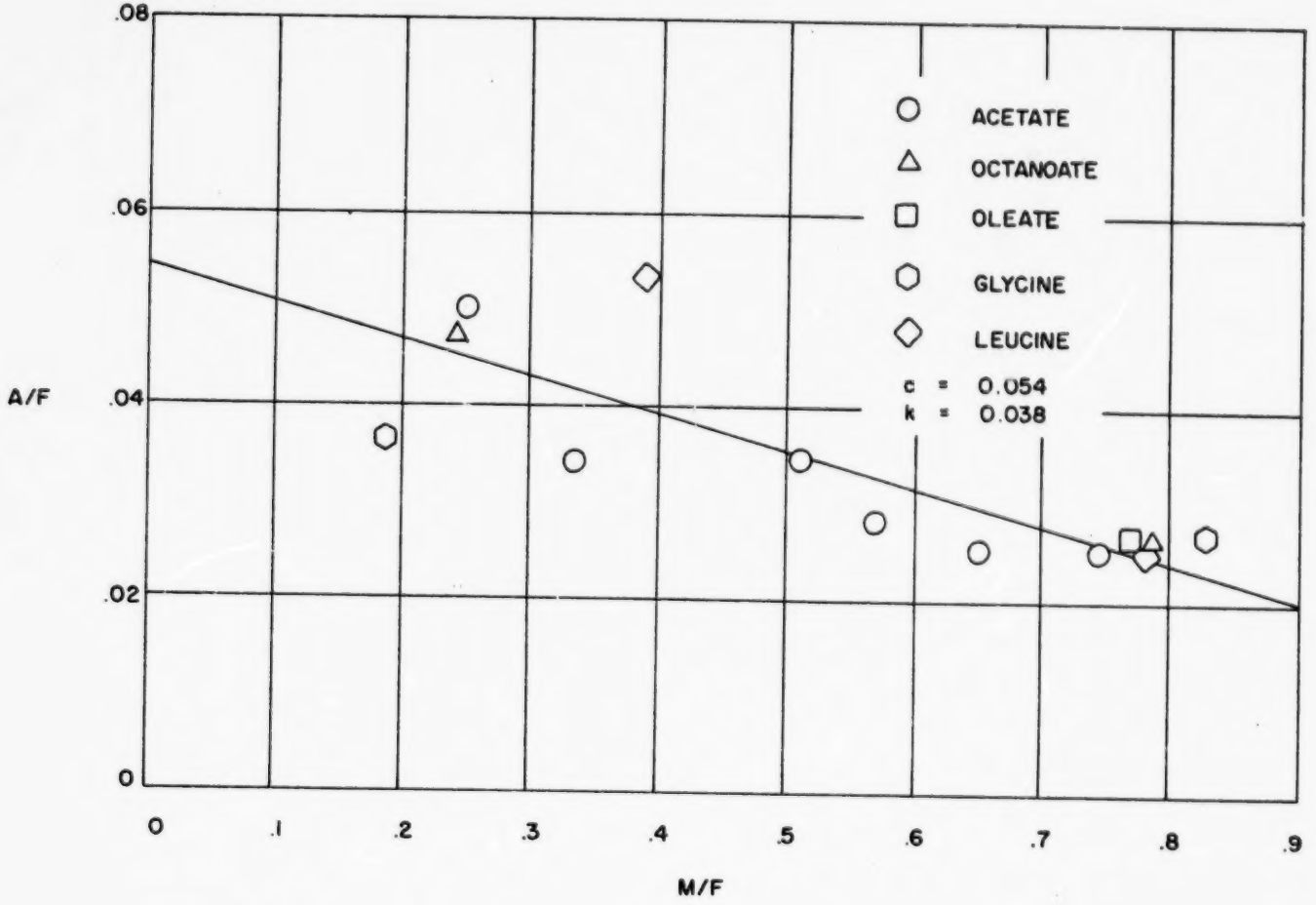

Figure 4. EVALUATION OF AMINO AND FATTY ACID GROWTH CONSTANTS

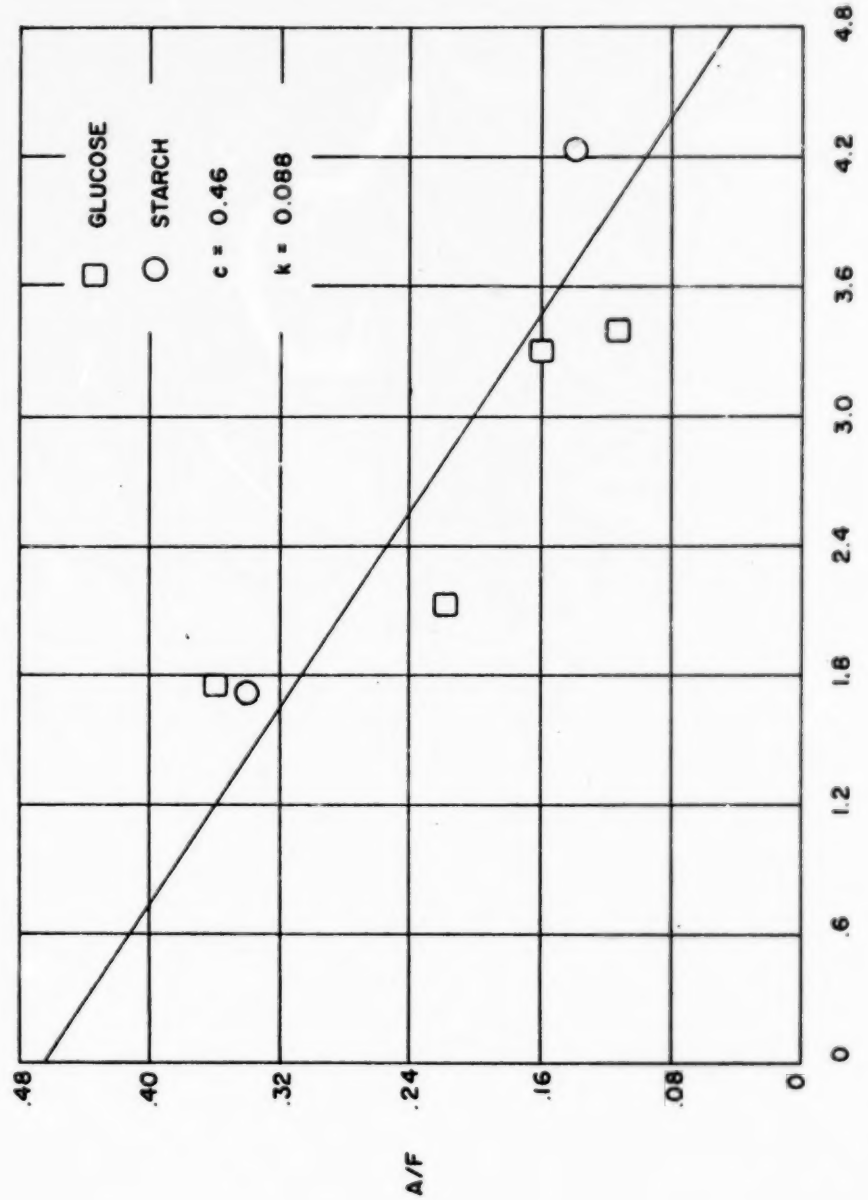

Figure 5 EVALUATION OF GLUCOSE AND STARCH GROWTH CONSTANTS

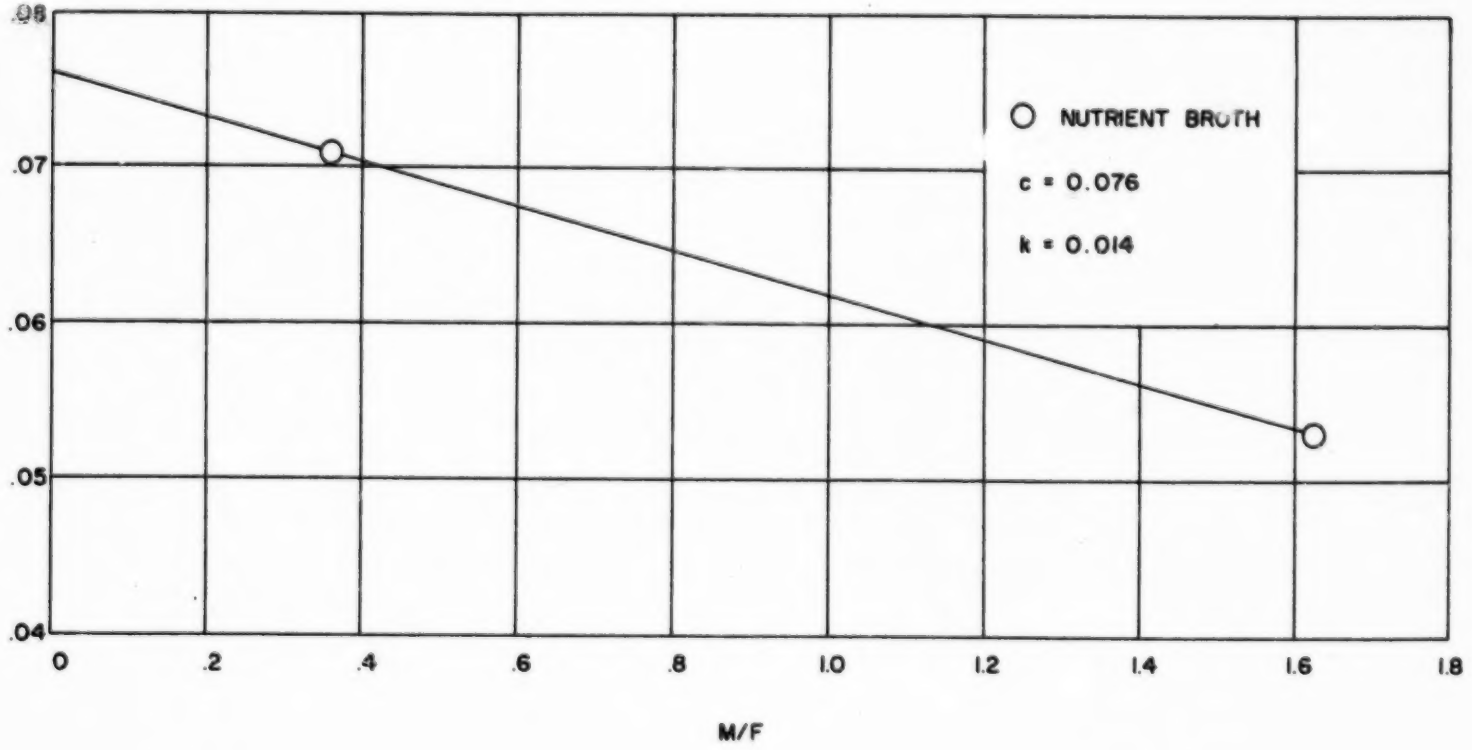

Figure 6. EVALUATION OF NUTRIENT BROTH GROWTH CONSTANTS

1. Short hydraulic detention times result in smaller digestion tank volumes which require less capital investment.

2. Long sludge retention times result in lower net synthesis, higher gas production and higher purification rates with the lower capital and operating costs involved therewith.

The Constant BOD and the BOD Reduction Stages

The anaerobic digestion process is often considered to take place in two stages. In the first stage, a multitude of facultative and anaerobic bacteria break complex organics into simple organic compounds such as fatty acids, alcohols, aldehydes, and ketones. In the second stage, these simple organics are then in turn converted to methane and carbon dioxide. The first stage of decomposition is brought about through hydrolysis and also oxidation and reduction of the complex organics. Since no inorganic oxidizing agent, such as oxygen is added and also no reduced organics are removed from solution, this first stage of change is brought about with no reduction in COD, and hence essentially no reduction in BOD. Minor exceptions to this would be when hydrogen gas is produced or when sulfates were used during the first stage as hydrogen acceptors.

The real COD and BOD reduction in anaerobic digestion occurs during the second stage of methane fermentation where the reduction is directly proportional to the methane gas production. This is true since no inorganic oxidant such as molecular oxygen is added to the system. Therefore, the only way a decrease in oxygen demand can be brought about is through the removal of a reduced compound from the system. The unique characteristic of the BOD reduction stage of digestion is that carbon dioxide, which is produced in the process, can serve as the hydrogen acceptor, being reduced to methane.

Conventionally, the essence of biological treatment of organic wastes is reduction in the oxygen demand. No such reduction is accomplished in the constant BOD stage of digestion. Yet, this is the stage which appears to exhibit the highest rates of net synthesis, with certain wastes, such as carbohydrates. Also, with carbohydrates, the decreased methane production is due to the high fraction of substrate synthesized in this constant BOD stage.

The bacteria responsible for the constant BOD stage are limited in the extent to which they can break down organic compounds. For instance, these bacteria can break down carbohydrates to simple acids and alcohols, but

The wastes most commonly encountered in anaerobic digestion have contained excess nitrogen and biologically resistant volatile solids. Also, sludge retention times have usually been 15 to 60 days. These conditions, plus the fact that net synthesis rates for substrates in the fats and proteins class have been shown to be quite low, have combined to mask the actual biological solids accumulation which occurs in anaerobic digestion.

The discovery of the stimulatory effect of iron on acetate utilization by methane bacteria proved to be a turning point in the progress of this study. Inclusion of the stipulated quantities of ferric chloride with the normal inorganic salts accomplished a universal reduction in the high concentration of volatile acids which had accumulated in the digesters. Acetate utilization was stimulated by the addition of iron filings as well.

Buswell and Sollo[4] found the presence of an inert surface within the digester aided digestion. However, this stimulation phenomena cannot be completely explained on the sole basis of an inert surface being introduced to the digester. The cellulose digesters provide a case in point where abundant surface area was present and still unsatisfactory digestion resulted as the original seed sludge was purged. The cellulose was fed in the form of shredded filter paper to which the bacteria could attach since it was not being decomposed readily. However, inclusion of ferric chloride in the daily feed resulted in a doubling of the gas production from approximately 150 to 300 ml/l of digester/day. The volatile acids concentration which had reached and steadily remained near 2000 mg/l was reduced to about 600 mg/l in 10 days and eventually dropped to 150 mg/l later on.

Figure 3 reveals a very marked decrease in nitrogen requirements for carbohydrates as the sludge retention time was increased, due to conservation of nutrients as a result of endogenous respiration. Nutrient broth exhibited a higher rate of net synthesis than the other two substrates in the protein class.

Many investigators[5], [14], [16] have operated digesters on short hydraulic detention times and long sludge retention times by retaining the biological solids in the digester through their recovery from the effluent and recycle back to the digester. Two advantages are thus achieved:

unable to carry the degradation further under anaerobic conditions. This step is performed by the methane bacteria. Therefore, direct feeding of these simple organic compounds results in the virtual absence of this constant BOD stage population of bacteria, since no food source is available to sustain them.

In the BOD reduction stage, a population of methane bacteria, which is apparently proportional to the BOD of the waste, degrades the simpler organic compounds. Since all substrates in this study were fed at the same loading rate, based on COD, all digesters on the same sludge retention time should therefore have contained comparable populations of methane bacteria.

Attention is now drawn to Figure 3 again. The rate of net synthesis for anaerobic decomposition of acetate is practically the same as for octanoate, oleate, glycine, and leucine. From this observation, the hypothesis is drawn that the bacterial population of the BOD reduction or methane fermentation stage of digestion is responsible for the major if not essentially the total decomposition of all these substrates.

Next, carbohydrate digestion would likewise require a comparable population in the BOD reduction stage to acetate digestion since the loading rates were equal. The hypothesis is then drawn that the difference between the net synthesis rate for carbohydrates and that for acetate, for each particular detention time, was due to the bacterial population responsible for the first or constant BOD stage of digestion. Considering this to be true, growth equations were evaluated for the two stages of carbohydrate digestion, assuming the second stage of this deoomposition would be the same as for acetate. The results of this evaluation gave the following equations:

First Stage (constant BOD):

$$A_1 = 0.406\ F - 0.100\ M_1$$

Second Stage (BOD reduction):

$$A_2 = 0.054\ F - 0.038\ M_2$$

An interesting observation of endogenous respiration was made from the glucose digesters. After final data were recorded, all of the units were left standing with no feeding for nine weeks. The volatile solids concentrations in the units were analyzed at the end of the final data period, six weeks later, and nine weeks later. Table IV shows the results of the analyses at these intervals.

Thus, after 60 days with no feeding, from 75% to 81% of the volatile solids were still present. This represents an endogenous respiration rate of less than 0.005/day as compared to 0.088/day, observed for the case when feed was being administered.

TABLE IV

ENDOGENOUS RESPIRATION OF BIOLOGICAL SOLIDS
IN GLUCOSE DIGESTERS AFTER STOPPAGE OF FEED

Time Weeks	Volatile Solids Concentration mg/l Sludge Rentention Time - Days 5	10	20	30
0	620	990	1970	2080
6	540	900	1540	1580
9	500	800	1540	1560

It is hypothesized that just as the bacterial population in the constant BOD stage appeared not to be able to derive energy from the amino and fatty acid group due to the lack of a hydrogen acceptor, so also, in the absence of a reducible organic substrate, endogenous metabolism is considerably diminished. The bacteria appear not to be able to attack their own protoplasm without a hydrogen acceptor, other than CO_2, present.

CONCLUSIONS

From this study on the biological nutrient requirements and growth in anerobic digestion, the following conclusions have been drawn:

1. From the results of this study, it is possible to predict for a given substrate, the nitrogen requirements, biological solids accumulation, net synthesis, and methane production which will occur during anaerobic digestion for various sludge retention times.

2. Two stages exist in the anaerobic digestion of complex substrates, one in which the BOD remains constant and another in which the BOD is reduced due to production of methane.

3. In the case of carbohydrates the major nutrient requirement and major biological solids accumulation result from the first or constant BOD stage of digestion.

4. The growth equation for anaerobic digestion of glucose and starch was: $A = 0.46\ F - 0.088M$.

5. The growth equation for anaerobic digestion of amino and fatty acids was: $A = 0.054F - 0.038 M$.

6. The growth equation for anerobic digestion of nutrient broth was: $A = 0.076 F - 0.014 M$.

7. The nitrogen requirements for all substrates were equal to A/9.4.

8. The phosphorous requirements for all substrates were approximately one-seventh of the nitrogen requirements.

9. Exceptionally high rates of acetate digestion, 6500 mg/l/day (0.36 lb/ft^3/day), can be achieved by addition of combinations of iron, cobalt, thiamine, and components of vitamin B_{12} to digesters purged of the original seeding material.

10. The addition of inorganic salts alone with the pure organic substrates studied in this investigation enabled satisfactory digestion.

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